Phycoerythrin Signatures In The Littoral Zone

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LONG-TERM GOALS

I am interested in the role of different phytoplankton species or taxonomic groups in ecosystem-level processes in the sea. Marine phytoplankton have evolved an astonishing array of different light harvesting systems and I am particularly interested in the way that the *in situ* optical environment determines which type of light harvesting system is most successful in a given optical environment. To this end, I have been working with experts in remote sensing and ocean optics to develop and "optical biogeography" for different spectral forms of marine picoplankton. These are globally important organisms for which my long-term goal is the development of methods to estimate their importance in a given region of the ocean by remote sensing. I am also interested in expanding the application of "optical biogeography" to other taxonomic groups of phytoplankton and to the question of whether or not water masses can be considered discrete habitats for microbial communities.

OBJECTIVES

Phycoerythrin (PE) is the principal light-harvesting pigment of marine *Synechococcus*, *Trichodesmium*, and red algae and a minor component of some strains of *Prochlorococcus*. It is a highly fluorescent molecule that absorbs light in the blue-green and green regions of the spectrum and emits light in the orange or yellow regions of the spectrum. Because these excitation/emission properties are so different from that of chlorophyll or most colored dissolved organic material (CDOM), it is relatively easy to obtain a distinct phycoerythrin spectral signature from a complex mix of colored material in a bulk seawater sample (Fig. 1). A variety of spectral forms of PE are found in *Synechoccocus* and *Trichodesmium*; these vary as a result of differences in the number and composition of the chromophores associated with the PEs synthesized by the cells. Each PE molecule is composed of a colorless protein heterodimer to which five or six chromophores are covalently bound. All PEs contain at least one phycoerythrobilin chromophore (PEB, λ_{AbsMax}~550 nm) but many extend their absorption into the blue regions of the spectrum by substitution of phycouroblin chromophores (PUB, λ_{AbsMax}~500 nm) at one or more of the other binding sites. This leads to a wide variety of spectral forms, each with greater or lesser efficiency at harvesting the green and blue-green wavelengths that penetrate coastal waters. Because PEs are water-soluble and do not co-extract with chlorophyll and most other photosynthetic pigments in organic solvents, little is known about its distribution or spectral variation in natural waters.

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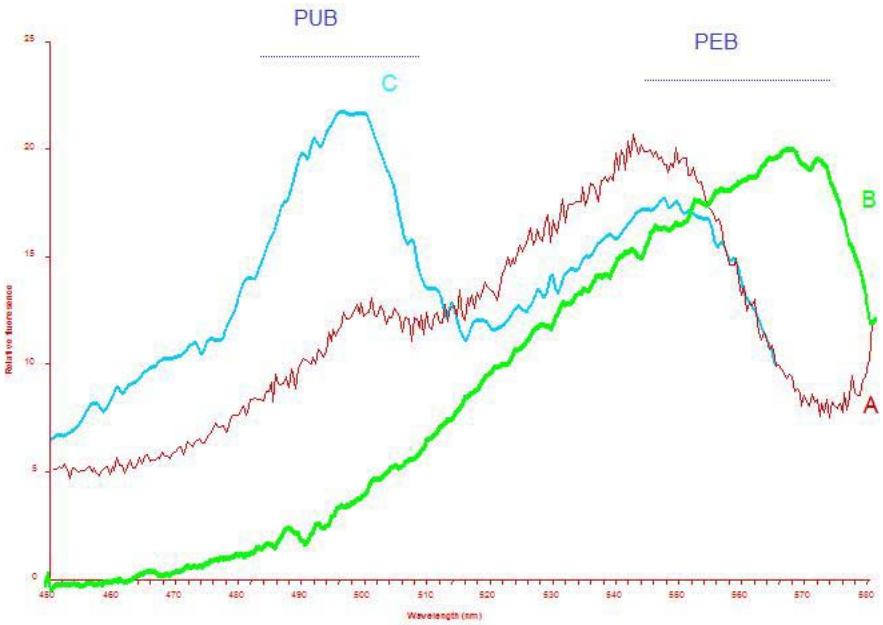

Figure 1. Quantum corrected excitation spectra for fluorescence emission from phycoerythrin in bulk seawater samples collected at the LEO-15 site off New Jersey. The spectrum typical of water dominated by organisms synthesizing high PUB-PEs(blue curve) has a peak at 500 nm that is higher than the peak at 550 nm; the ratio of these two peaks is the Ex_{PUB}:Ex_{PEB} ratio. For high PUB spectra, the ratio is greater than one. The spectrum from a sample dominated by low-PUB PEs (red curve) has an Ex_{PUB}:Ex_{PEB} ratio around 0.5; the spectrum has a peak at 500 nm and a shoulder at 550 nm. PE excitation spectra from a sample dominated by PUB-lacking PEs (green curve) has a peak at about 560 nm and very little evidence of excitation around 500 nm. If an Ex_{PUB}:Ex_{PEB} were calculated for this spectrum it would be about 0.2.

PEB provides for efficient utilization of green wavelengths of light and PUB enhances absorption of blue and blue-green wavelengths of light, which penetrate clear ocean water more effectively. The cyanobacteria, which utilize PE as the principal light harvesting pigment, are under very strong selection to synthesize a pigment with a chromophore composition that can efficiently utilize the available wavelengths (Wood, 1985). Thus, it has generally been assumed that the high PUB form of PE dominated in the ocean. However, preliminary work from the Arabian Sea and North Atlantic suggested that, when nutrient input led to increased chlorophyll concentration or CDOM increased the attenuation of blue light, the low PUB or PUB-lacking forms of PE might predominate (Wood et al. 1998, 1999). This project has two main goals.

The first goal is to test the hypothesis that different spectral forms of PE predominate in Case II waters, "blue" Case I waters [$K_d(440)<K_d(550)$], and "green" Case I waters [$K_d(440)>K_d(550)$]. Specifically, I hypothesized that the PUB-lacking form of PE would predominate in Case II waters, the low PUB form of PE would predominate in "green" Case I waters, and the high PUB form of PE would predominate in "blue" Case I waters. A second objective of the project is to examine the biological basis for differences in PE spectral signature of a given water mass. We are examining the likelihood

of two different potential mechanisms. First, changes in PE spectral signature may result from changes in the species compositions of the community. These populations are usually large and have short generation times; this provides considerable opportunity for a change in genotype frequency in response to selection (Sherry et al., 2001; Wood 1990). Second, changes in PE spectral signature may reflect a physiological adaptation by which a single genotype or species is able to change the spectral form of PE that is synthesized. At least some PE-containing marine picocyanobacteria contain multiple copies of PE with different chromophore composition (Wilbanks et. al., 1991), so one way that strains could adapt to a changing optical environment is by differential expression of the two copies of PE.

APPROACH

This work has two major components. The approach to the first objective has been to participate in cruises where other investigators were already funded to collect in-water optical data and to provide remote sensing support. My lab measures phycoerythrin spectral signature, the abundance of PE-containing organisms, and collects other data necessary to understand the correlation of the PE spectral signature with the optical environment and its determinants. Our second goal has been to understand the biological mechanisms for changes in the PE spectral signature of a water mass. In other words, does the PE spectral signature that predominates in a water mass change because the genetic makeup of the community changes or because the organisms present are capable of a physiological adaptation to the changing light regime? If the PE spectral signature of a water mass changes as a result of a change in genetic composition, then the communities must be genetically diverse to begin with. Thus, one experimental approach we are using to address our second objective is to examine the genetic diversity within the communities. We are also using molecular genetic approaches to examine the genetic architecture of the region of the genome that codes for the different phycoerythrins. Working with a wide range of isolates of PE-containing picocyanobacteria we are able to examine the number of genetic bases for differences in spectral signature. Finally, we are screening a large number of cultures for their ability to change spectral signature by growing them under conditions where the spectral composition of available irradiance is modified using theatrical gel filters. We plan to use real-time PCR to look at the expression of PE genes in any chromatically adapting strains we do identify and to examine the expression of PE genes in strains which adjust the Ex_{PUB}:Ex_{PEB} ratio in reponse to irradiance levels in white light. We have observed diurnal changes in the ratio in the field (Sherry and Wood, 2002), which makes this work particularly interesting.

WORK COMPLETED

We have completed the fieldwork required to address our first objective. The data for this project come primarily from six cruises, one on the West Florida shelf, two in the northern Gulf of Mexico, one in the Gulf of California, one off New Jersey, and one off of Monterrey Bay. The final cruise (Monterrey Bay, 2003) provided our only upwelling system for this project and served to confirm observations made in the upwelling system of the Arabian Sea during a previous ONR funded project. Preliminary analysis of the data from all cruises has been completed and presented at major scientific meetings. We have begun integration of the biological data with all the optical data from all six cruises in preparation for writing a major synthetic paper on PE spectral signatures in the coastal ocean. On most cruises a complete set of optical data was collected including hyperspectral measurements of upwelling radiance and downwelling irradiance, dissolved and total beam attenuation (ac-9, filtered and unfiltered; ac-100 on some cruises), chlorophyll fluorescence, turbidity, CDOM fluorescence, and in all but one case, we had excellent satellite coverage for the study area. All of the

scientists who provided these data have expressed interest in continuing the collaboration to gain the maximum insight we can into the optical environment and optical impact of these PE-containing cells.

The lab has developed a large collection of new isolates of PE-containing organisms and we have modified enrichment techniques so that we are accumulating a representative sample of all spectral types of strains. We also have developed a good capacity for routine amplification of target genes and phylogenetic analysis based on 16S rRNA sequences (Wood et al., 2002; Wingard et al. 2002). A Ph.D. student, Mr. Craig Everroad has nearly completed a study in which he examines the question of how much genetic diversity exists within a group of cultures isolates that share the same spectral phenotype. The results of this study are described below. In pursuing this work, Mr. Everroad has developed a suite of degenerate and non-degenerate primers that target conserved and nonconserved regions of both subunits of the PE gene. These primers range from "universal" primers designed to amplify all known PE genes to primers designed for specific PE genes(*cpe* or *mpe*, α or β subunits). Development of these primers represents a significant amount of work and provides us with a powerful set of tools for obtaining near complete sequence data for PE genes from most organisms.

RESULTS

Our fieldwork has shown that the PUB-lacking form of PE is restricted to Case II waters and that the high PUB-lacking form is found in "blue" Case I waters. Analysis of remote sensing data from our West Florida Shelf cruise has shown that, in regions where there are very high concentrations of PE-containing picocyanobacteria, CDOM is the principal determinant of ocean color (Wood et al., In Prep.). Further, the same low PUB spectral signature has dominated all upwelling influenced regions that we have studied, regardless of latitude and water temperature. This includes the Gulf of California, the Monterey Bay region, and the upwelling-influenced waters of the Arabian Sea. The fieldwork has also shown that PE-containing picocyanobacteria are much more abundant in nearshore coastal waters and nutrient-enriched oceanic waters than the oligotrophic open ocean. Finally, studies already completed by Mr. Everroad show that at least one set of picocyanobacterial strains from the Black Sea and Arabian Sea have a unique 16S ribosomal RNA sequence that indicates they are more closely related to other cyanobacteria in the genus *Cyanobium* than *Synechococcus* (Cluster 5.1) and that their PE, while similar to that of the PE in marine *Synechoccocus* (Cluster 5.1), also has unique architecture (Everroad et al. 2003).

IMPACT/IMPLICATIONS

Overall this work has tremendous implications for our understanding of the role of PE-containing picocyanobacteria in marine ecosystems. Because these organisms are dominant in much of the open ocean, they have generally been ignored in coastal waters. However, it is clear that this is their preferred niche and their exclusion from low light environments at the base of the euphotic zone in transparent waters is not because they are intolerant of low light. In fact, they appear to reach their highest abundance in turbid green water. It is not that they require high light, but they cannot tolerate low light if it is accompanied by a shift to monochromatic blue light. This appears to be what keeps them from reaching high density in the deep chlorophyll maximum. In turbid waters, where chlorophyll and CDOM cause a spectral shift in the optical environment to green wavelengths, PEB-rich PEs are favored and PE-containing organisms thrive. The results of our work with optical water mass classification based on remote sensing data products (Wood et al., In Prep.) indicates that high CDOM is an important environmental correlate for high concentrations of PE-containing organisms.

This suggests a possible relationship with CDOM as a source of nutrients, either directly or through its role as a chelator. This work is especially important because it highlights the meaningful new insights into the ecology and physiology of different phytoplankton groups that can emerge from coordinated application of remote sensing and biological oceanography.

RELATED PROJECTS

Optical measurements for this research were made by Scott Pegau on the Gulf of California and HyCODE cruises and by Bob Arnone and Rick Gould (NRL/SSC) on the other cruises. Remote sensing support has been provided by Bob Arnone, the OSU Ocean Optics group, and Jim Mueller (SDSU/CHORS). Pigment data have been provided by Steve Lohrenz (USMs), Chuck Trees (SDSU/CHORS), and Mark Molin (CalState). Funding for these investigators has come from ONR, NRL, and NASA SIMBIOS. On the Monterey Bay cruise, West Florida Shelf cruise, and HyCode cruises, we tried to coordinate sampling with aircraft overflights by Curt Davis' program (NRL). Analysis of samples by flow cytometry is being done through a long-standing collaboration with W. K. W. Li (Bedford Inst. Of Oceanography) and much of the financial support for that collaboration is coming from the Department of Fisheries and Oceans (Canada).

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